Villin Controls the Formation and Enlargement of Punctate Actin Foci in Pollen Tubes

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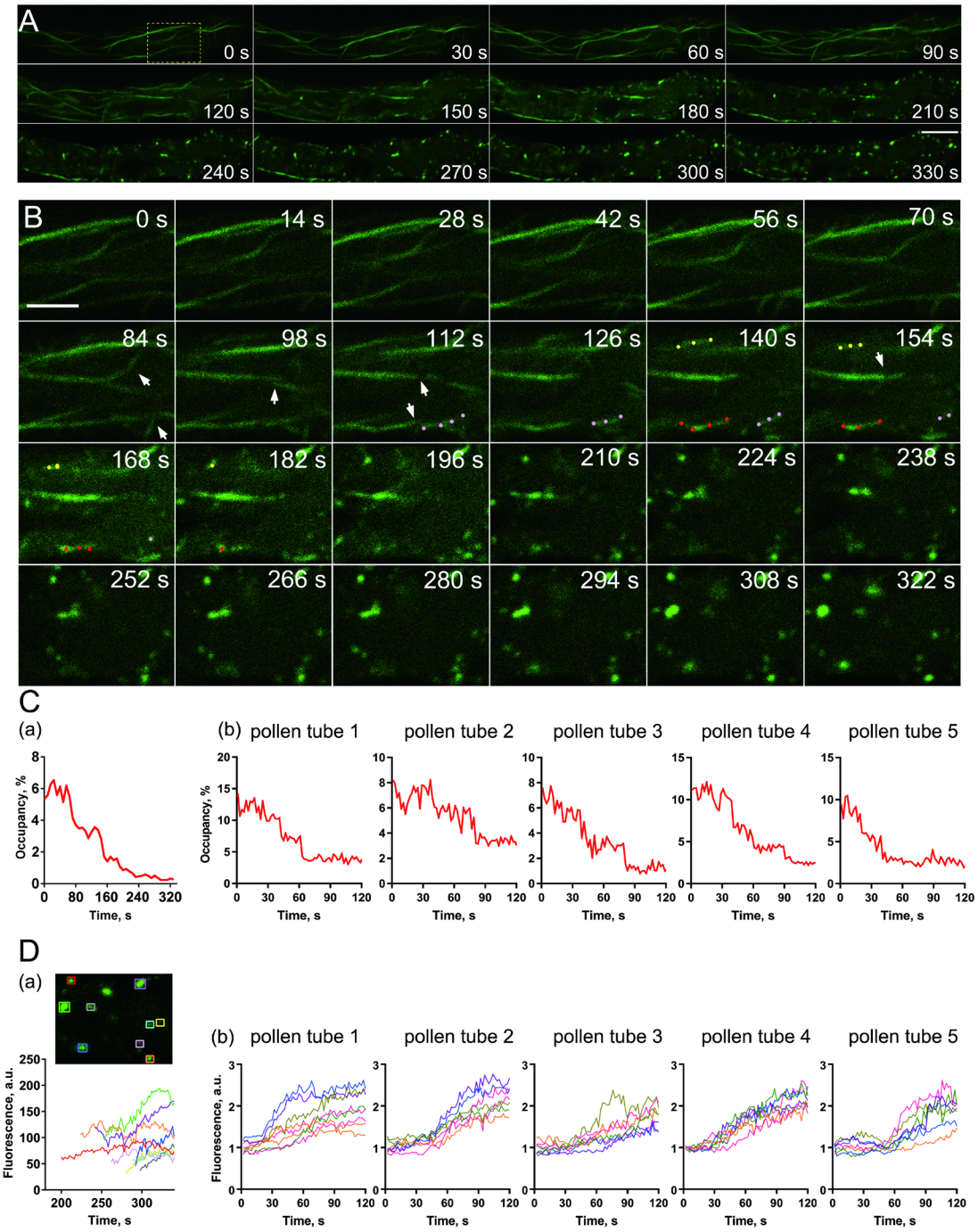

Figure S1. A23187 Treatment Induces Rearrangement, Fragmentation and Depolymerization of Actin Filaments as well as the Formation and Subsequent Enlargement of Actin Foci in Pollen Tubes

(**A**) Time-series of images showing remodeling of actin filaments in a living WT pollen tube expressing Lifeact-EGFP treated with 10 μM A23187. Actin filaments were revealed by decoration with Lifeact-EGFP. Bar = 5 μm. t = 0 represent the time

point when the rearrangement of actin filaments began.

(**B**) Time-series images of actin filaments within the boxed region shown in (**A**). The red, pink and yellow dots mark different actin filaments, which underwent apparent depolymerization during A23187 treatment. The white arrows indicate actin filament fragmentation events. Bar = 2 μm.

(**C**) Quantification of the occupancy of actin filaments in pollen tubes. (**a**) The occupancy of actin filaments was plotted versus the time after A23187 addition for the pollen tube shown in (**A**). (**b**) Plot of the occupancy of actin filaments versus the time after A23187 addition for 5 additional pollen tubes.

(**D**) Quantification of the fluorescence intensity of actin foci. (**a**) The upper panel shows the actin foci of interest traced for the measurement of the fluorescence intensity, which was indicated by different colored boxes. The lower panel shows the plot of fluorescence intensity of actin foci versus time; the fluorescence intensity of foci was recorded once the foci started to form. (**b**) Plots of fluorescence intensity of actin foci versus time for 5 additional pollen tubes.

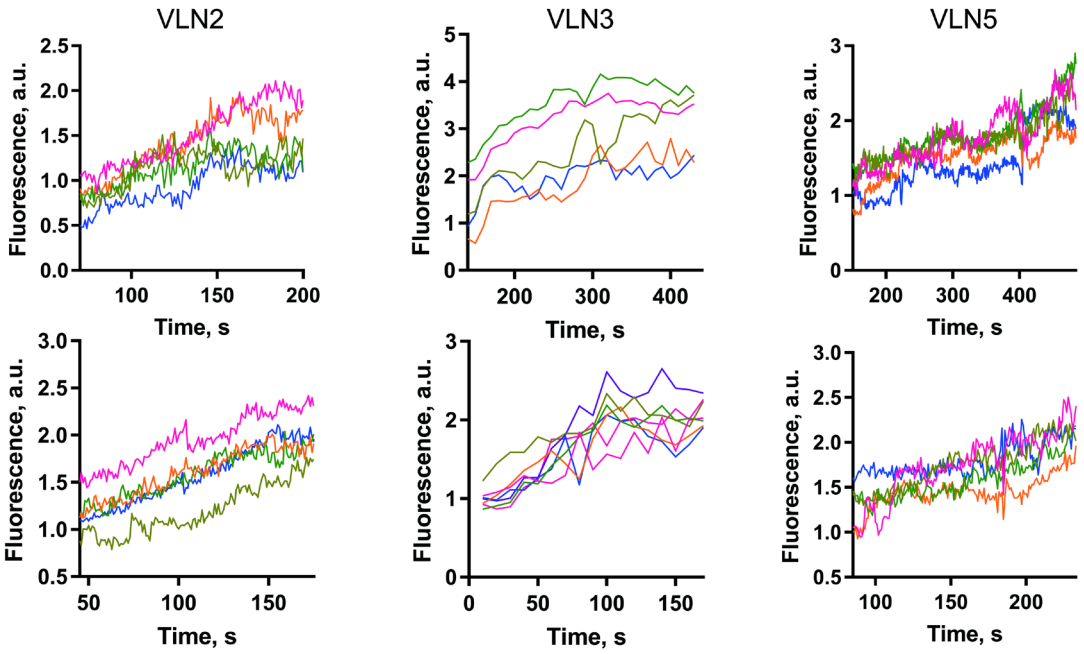

Figure S2. The Fluorescence Intensity of Actin Foci Decorated with VLN2-EGFP, VLN3-EGFP and VLN5-EGFP Increases Over Time

The fluorescence intensity of dots formed by VLN2-EGFP, VLN3-EGFP and VLN5-EGFP was measured in two pollen tubes for each GFP fusion protein, and was plotted versus time.

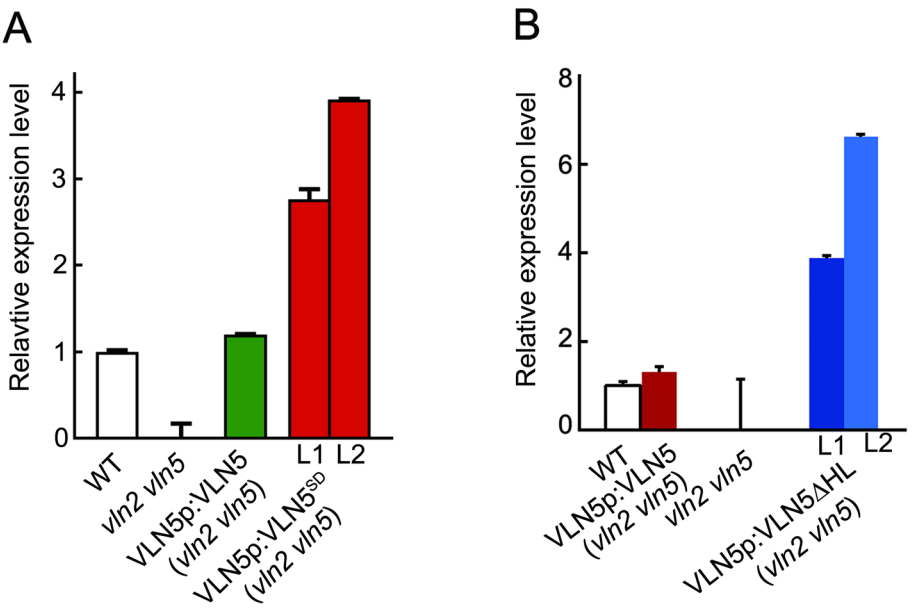

Figure S3. Determination of the Relative Amount of Transcripts of VLN5 and Its Variants in Pollen

(**A**) Analysis of the amount of *VLN5* transcripts in pollen. Pollen derived from WT, *vln2 vln5*, VLN5p:VLN5 (*vln2 vln5*), VLN5p:VLN5^{SD} (*vln2 vln5*) plants. VLN5p:VLN5 (*vln2 vln5*) are transgenic plant lines expressing *VLN5* under control of *VLN5* promoter in *vln2 vln5* double mutant.

(**B**) Analysis of the relative expression level of *VLN5* transcripts in pollen from various lines. Pollen was collected from WT, *vln2 vln5*, VLN5p:VLN5 (*vln2 vln5*), VLN5p:VLN5ΔHL (*vln2 vln5*) plants; see M&M for details.

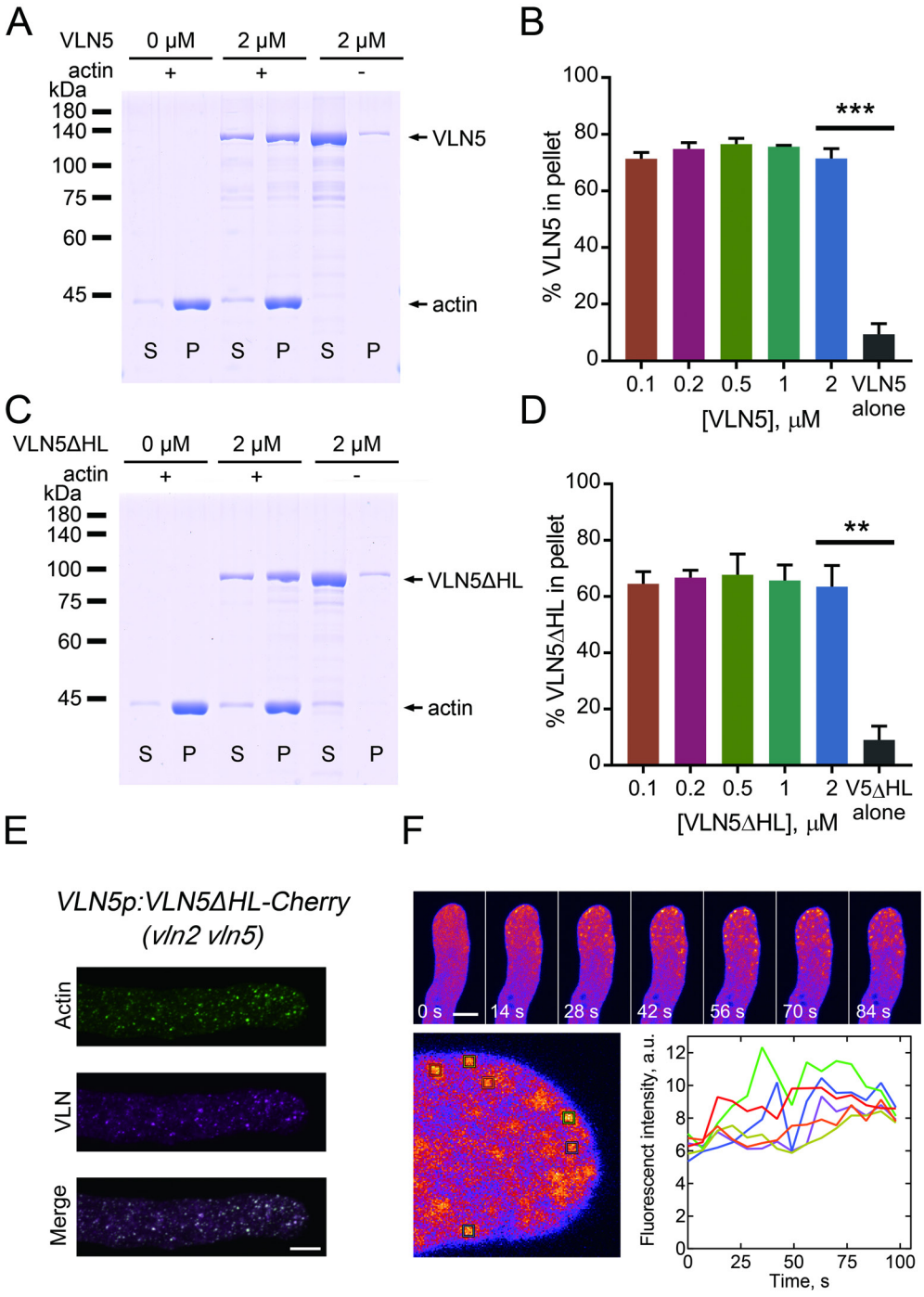

Figure S4. Actin Foci Decorated by VLN5ΔHL Fail to Enlarge

(**A**) SDS-PAGE analysis of the protein samples from the high-speed F-actin cosedimentation experiment. 2 μM of VLN5 (with 0 μM as control) was incubated with 3 μM F-actin for 30 min at room temperature before centrifugation. S, supernatant; P, pellet.

(**B**) Quantification of the amount of sedimented VLN5. The amount of VLN5 in pellet significantly increased after incubation with F-actin, indicating that VLN5 possesses F-actin binding ability. Values represent mean ± SE, n = 3. ***P < 0.001 by Student's t-test.

(**C**) SDS-PAGE analysis of the protein samples from the high-speed cosedimentation experiment. 2 μM of VLN5ΔHL (with 0 μM as control) was incubated with 3 μM F-actin for 30 min at room temperature before centrifugation. S, supernatant; P, pellet.

(**D**) Quantification of the amount of sedimented VLN5ΔHL. The amount of VLN5ΔHL in pellet significantly increased after incubation with F-actin, indicating that VLN5ΔHL possesses F-actin binding ability. Values represent mean ± SE, n = 3. **P<0.01, by Student's t-test.

(**E**) Images of actin and VLN5ΔHL-mCherry in *A. thaliana* pollen tubes after treated with A23187. Actin filaments were revealed by staining with Alexa-488 phalloidin. Actin foci and VLN5ΔHL-mCherry-decorated structures overlap substantially. Bar = 5 μm.

(**F**) Quantification of the dynamic behavior of VLN5ΔHL-mCherry in pollen tubes after treatment with A23187. The upper panel shows time-lapse images of VLN5ΔHL-mCherry in pollen tubes treated with 10 μM A23187. The lower panel shows the tracked VLN5ΔHL-mCherry dots (left) and quantification of their fluorescence intensities over time (right). Bar = 5 μm.

Supplemental Movies

Movie 1. Dynamic Behavior of Actin Filaments in Pollen Tube Treated with 10 μM A23187. Actin filaments decorated with Lifeact-EGFP were tracked after the treatment with 10 μM A23187. The movie is displayed at 5 frames per second.

Movie 2. Dynamic Formation of VLN2-EGFP Dots in a Pollen Tube after the Treatment with 10 μM A23187. VLN2-EGFP was tracked after the treatment with 10 μM A23187 showing the formation of dot-like structures. The movie is displayed at 5 frames per second.

Movie 3. Dynamic Formation of VLN3-EGFP Dots in a Pollen Tube after the Treatment with 10 μM A23187. VLN3-EGFP was tracked after the treatment with 10 μM A23187 showing the formation of dot-like structures. The movie is displayed at 2 frames per second.

Movie 4. Dynamic Formation of VLN5-EGFP Dots in a Pollen Tube after the Treatment with 10 μM A23187. VLN5-EGFP was tracked after the treatment with 10 μM A23187 showing the formation of dot-like structures. The movie is displayed at 5 frames per second.

Table 1. Primers used in this study

Primer name	Primer sequence
$VLN5CDS_F$	GGTACCGAGCTCCGATGACGTTTTCCATGAG
$VLN5CDS_R$	CTGCAGCTTCAGTTGCGCTC
$VLN5^{SD}{}_{1F}$	ACTATTAGGTGAACGTGCTGTTC
$VLN5^{SD}{}_{1R}$	GTATTTAAATCAACAGTCATGACGGC
$VLN5^{SD}{}_{2F}$	CACAACACTTCAAAGCCCGAGGA
$VLN5^{SD}{}_{2R}$	GAACGCTGAAGCAACTCCACCT
$VLN5\Delta HL_F$	GGTACCGAGCTCCGATGACGTTTTCCATGAGAGATTT
$VLN5\Delta HL_R$	GAATTCTTACTCGAGATTTGTCAGGATCGCAAGCTTTC
q*VLN5* F	TCGGTAAAGATTCCAGCCA
q*VLN5* R	GAACCCTGAAGCAACTCCAC